SPECIFICITY OF STREPTOCOCCI ISOLATED FROM PATIENTS WITH SKIN DISEASES: STUDIES ON PEMPHIGUS, DERMATITIS HERPETIFORMIS, LUPUS ERYTHEMATOSUS AND ERYTHEMA MULTIFORME*

II. Dermatitis Herpetiformis

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The theory of an infectious etiologic agent in dermatitis herpetiformis has many advocates. To date no conclusive proof of this theory has been established. The investigations described here support the specificity of a streptococcus isolated from patients who had dermatitis herpetiformis.

E. Riecke (1), in his monograph on dermatitis herpetiformis, has reviewed the literature on the etiology of this disease up to 1931. Analysis of his review reveals the fact that no conclusive proof of any theory of the genesis of this disease had been established up to that date. Since that time, as in the case of pemphigus, little of significance has been recorded in support of any theory except the infectious one. These investigations have been aimed at establishing a filtrable virus as the etiologic agent in Duhring's disease and pemphigus. A review and critical analysis of the experiments recorded to date in support of the virus etiologic theory of these two diseases, together with a description of my unsuccessful attempts at duplication of certain of these investigations is included in earlier publications (2, 3).

From an analysis of the evidence reviewed by E. Riecke and the investigations just mentioned, it is apparent that as yet no conclusive proof exists for any of the various etiologic theories suggested for dermatitis herpetiformis.

BACTERIOLOGIC STUDIES

This report deals with the bacteriologic studies of the blood, of material from the nasopharynx, of fluid from blisters, of cerebrospinal fluid, and of material from the apices of infected teeth, infected tonsils and prostatic secretion obtained from thirty patients who had dermatitis herpetiformis. The cultural results obtained are recorded in Table 1. A total of sixty-nine pure cultures of a characteristic streptococcus was isolated from the above sources from these patients. The technic employed in every procedure in these investigations on dermatitis herpetiformis was identical in every detail to that already described as employed in similar studies on pemphigus (2, 3).

CHARACTERISTICS OF THE STREPTOCOCCUS

The morphology and staining characteristics of the streptococci isolated from patients who had dermatitis herpetiformis is similar and indistinguishable from these properties of green-producing streptococci isolated from normal throats.

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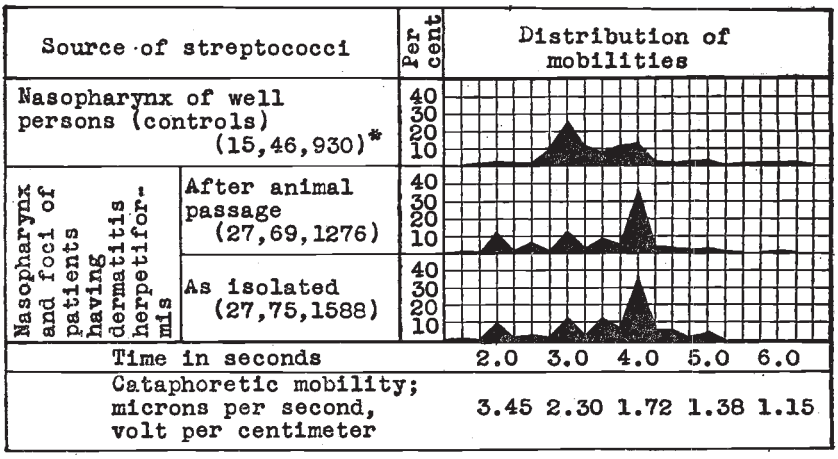
Cultural characteristics. This organism grows equally well at partial tension of oxygen and anaerobically. It grows best in Rosenow's (4) dextrose-brain

TABLE 1

Cultural results obtained in thirty patients who had dermatitis herpetiformis

NUMBER OF	MATERIALS CULTURED						
	Swabbings from Naso-pharynx	Blood	Blister-fluid	Cerebro-spinal fluid	Foci		
					Teeth	Tonsils	Pros-tatic fluid
Patients from whom material was studied	30	12	10	4	1	3	1
Patients from whom characteristic streptococcus was recovered	29	0	0	0	1	3	1
Attempts to recover characteristic streptococcus	53	12	77	4	1	3	1
Attempts resulting in recovery of characteristic streptococcus	43	0	0	0	1	3	1
Pure cultures of characteristic streptococcus obtained	63	0	0	0	2	3	1

A total of sixty-nine pure cultures of a characteristic streptococcus were isolated from the above sources from these thirty patients who had dermatitis herpetiformis.



*The figures in parenthesis indicate respectively, the number of strains, cultures, and streptococci timed in each group.

FIG. 1. Distribution curves of the cataphoretic time and mobility of streptococci isolated from the nasopharynx and foci of patients suffering from dermatitis herpetiformis.

broth. In this medium growth first appears at the bottom of the tube and later becomes fairly diffuse. Sediment is seldom present but if it does appear, is flocculant. Colonies of this organism on blood agar plates are indistinguishable

from the colonies of green-producing streptococci usually found in throats except that they produce more greening and often, at the same time, slight hemolysis. Optimal growth occurs at 35° C.

Fermentation reactions. The fermentation reactions of fresh strains of the streptococcus as determined in peptone broth with bromthymol blue as the indicator paralleled exactly those described for the streptococcus from pemphigus, except that raffinose was regularly fermented by the former streptococcus but less readily by the latter.

Determinations of cataphoretic mobility. The two peaks (1.72 and 3.45) in the cataphoretic mobility distribution curve (fig. 1) of streptococci which were isolated from the nasopharynges and foci of infection of patients who had dermatitis herpetiformis, as determined by means of the Mudd assembly of the Northrop-Kunitz apparatus, were the same as those already described for the streptococcus from pemphigus (2, 3) and for other streptococci (5, 6, 7) having elective localizing properties for ectodermal tissues.

INJECTION INTO ANIMALS

The organism was injected into a total of 229 mice, rats, guinea pigs and rabbits. It was virulent, producing death in from eight to thirty-six hours in animals of all four species, when injected intracerebrally or intravenously when the amounts given were comparable to those of other green-producing streptococci usually given to the same animals by the same routes. In contrast to the streptococcus isolated from patients who had pemphigus this organism was not especially virulent for mice. Intravenous injection into rabbits of 0.5 cc. per 100 grams of body weight of a suspension, turbidity[1] 500, of the primary or early, rapid subculture, killed by heating, of streptococci from patients who had dermatitis herpetiformis, produced hermorrhages in the skin of about 70 per cent of these animals. Control strains of green-producing streptococci isolated from patients with arthritis, myositis, tinea corporis and from normal persons failed to produce these lesions. No attempt was made to reproduce the clinical picture of dermatitis herpetiformis in animals with this organism.

EXPERIMENTS TO ESTABLISH THE IDENTITY OF THE INDIVIDUAL STRAINS[2] OF THE STREPTOCOCCUS

Agglutination reactions with immune horse and immune rabbit serums. The titer of agglutinins in the serums of patients who had dermatitis herpetiformis was found to be insufficient to demonstrate clearly cross agglutination of the different strains of the streptococcus. Therefore, each of eleven rabbits was immunized with a single strain and a horse (104) was immunized with a mixture of nine strains of this streptococcus.

[1] U. S. Government turbidity standard, hereafter abbreviated to T.

[2] In order to avoid confusion, the word "strain" in the remainder of this paper is applied to each of the individual pure cultures of the organism isolated from patients who had dermatitis herpetiformis. It is used in the same manner in referring to streptococci isolated from patients with other diseases.

As is indicated in Table 2, each of the immune rabbit serums and the immune horse serum agglutinated to some degree all, and to a marked degree most, of the fifteen strains of the streptococcus from patients who had dermatitis herpetiformis

TABLE 2

Agglutination Reactions

Individual serums from eleven rabbits immunized with single strains, and the serum of a horse (104) immunized with a pool of nine strains of streptococci isolated from patients with dermatitis herpetiformis, agglutinate fifteen strains of streptococci from other patients with the same disease

STREPTOCOCCI FROM PATIENTS WITH:	STRAIN NUMBER	INDIVIDUAL SERUMS FROM RABBITS IMMUNIZED TO SINGLE STRAINS OF STREPTOCOCCI ISOLATED FROM PATIENTS WHO HAD DERMATITIS HERPETIFORMIS (DILUTION 1:20)											SERUM OF IMMUNE HORSE 104*	CONTROL SERUMS (1:20)				SALT SOLUTION
		Rabbit												Immunized to streptococci from pemphigus		Normal		
		468	469	470	471	474	475	476	477	478	479	480		Rabbit	Horse	Rabbit	Horse	
Dermatitis Herpetiformis (Single strains)	1	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	1+	1+	1+	1+	1+
	2	4+	3+	3+	4+	3+	4+	4+	3+	4+	4+	3+	4+	−	−	−	−	−
	3	1+	1+	2+	1+	1+	1+	1+	3+	2+	3+	4+	4+	−	−	−	−	−
	4	3+	4+	4+	4+	4+	4+	4+	3+	3+	4+	4+	3+	−	−	−	−	−
	5	4+	4+	2+	3+	3+	4+	4+	4+	3+	4+	4+	4+	−	−	−	−	−
	6	1+	1+	1+	1+	1+	1+	2+	2+	3+	2+	2+	4+	−	−	−	−	−
	7	1+	2+	2+	1+	2+	3+	4+	2+	3+	2+	3+	3+	−	−	−	−	−
	8	2+	2+	3+	1+	4+	3+	2+	2+	3+	3+	3+	2+	−	−	−	−	−
	9	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	−	1+	−	1+	1+
	10	2+	2+	3+	3+	3+	2+	3+	3+	4+	3+	2+	3+	−	−	−	−	−
	11	3+	3+	4+	2+	3+	3+	4+	3+	2+	3+	2+	2+	−	1+	−	1+	−
	12	1+	3+	4+	1+	2+	3+	2+	1+	1+	2+	1+	2+	−	−	−	−	−
	13	3+	3+	2+	3+	4+	4+	2+	2+	3+	4+	2+	4+	−	−	−	−	−
	14	2+	4+	3+	4+	4+	4+	3+	2+	3+	4+	2+	3+	−	−	−	−	−
	15	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	3+	1+	−	1+	−	−
Pemphigus (Pool of 19 Strains) #102		−	−	−	−	−	1+	−	−	−	−	−	−	2+	3+	−	−	−
Lupus erythematosus (Pool of 7 strains) #104		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Erythema multiforme (Pool of 7 strains) #132		−	−	−	−	−	1+	−	−	−	−	−	−	−	−	−	−	−

* This horse was immunized with a pool of nine strains of streptococci derived from patients who had dermatitis herpetiformis.

but failed to agglutinate the control streptococci from pemphigus, lupus erythematosus and erythema multiforme, except the serum from rabbit (475) which agglutinated slightly (1+) the control strains from pemphigus and from erythema multiforme. A control pool of immune rabbit serums and an immune horse

serum as well as normal rabbit and normal horse serum agglutinated a few of these strains slightly and two were autoagglutinable in physiologic solution of sodium chloride.

After demonstrating cross agglutination with each immune rabbit serum, a pool was made of equal quantities of the eleven serums. This pool of immune rabbit serums and the serum from a horse (104), after immunization for more

TABLE 3

Agglutination reactions between single strains of the streptococcus isolated from patients who had dermatitis herpetiformis and pooled immune rabbit serums and immune horse serums

STREPTOCOCCI FROM PATIENTS WITH:	STRAIN NUMBER	HORSE SERUM (1:2000)					POOLED RABBIT SERUM (1:200)					SALT SOLUTION CONTROL
		Horse 104 immunized with nine strains from dermatitis herpetiformis	Horse 99 immunized with nineteen strains from pemphigus	Horse 103 immunized with eight strains from lupus erythematosus	Horse 105 immunized with seven strains from erythema multiforme	Normal	Immunized with seven strains from dermatitis herpetiformis	Immunized with seven strains from pemphigus	Immunized with eight strains from lupus erythematosus	Immunized with five strains from erythema multiforme	Pooled normal	
Dermatitis Herpetiformis* (Single strains)	19	4+	−	−	−	−	3+	−	−	−	−	−
	20	4+	−	−	−	−	4+	−	1+	−	−	−
	21	4+	2+	1+	1+	−	3+	1+	−	1+	−	−
	22	4+	1+	−	1+	−	3+	−	−	−	−	−
	23	4+	−	−	−	−	3+	−	−	−	−	−
	24	3+	1+	1+	−	−	4+	2+	1+	1+	−	−
	25	4+	1+	1+	1+	1+	3+	−	1+	1+	−	−
	26	3+	−	−	−	−	2+	−	−	−	−	−
	27	4+	1+	1+	1+	−	3+	1+	−	−	−	−
	35	4+	−	−	−	−	3+	−	−	−	−	−
Pemphigus (Single Strain)	5	−	4+	−	−	−	1+	4+	1+	−	−	−
Lupus erhthematosus (Single Strain)	6	−	1+	4+	−	−	−	1+	4+	−	−	−
Erythema Multiforme.... (Single Strain)	8	−	−	1+	4+	−	−	1+	1+	4+	−	−
Arthritis (Pool of six strains) #5418	−	−	−	−	−	−	−	−	−	−	−	−

* Twenty five other single strains isolated from patients who had dermatitis herpetiformis gave approximately the same results as the ten recorded above.

than one year, were used as agglutinating serums to test other individual heterologous strains isolated from patients who had dermatitis herpetiformis.

As is shown in Table 3, the serum from horse (104) and the pool of immune rabbit serums described above agglutinated each of ten individual heterologous strains of streptococci from dermatitis herpetiformis. It is also shown that the control immune serums from horses (99), (103) and (105) immunized (for more than one year) with streptococci from pemphigus, lupus erythematosus and erythema multiforme respectively and control pooled serums from rabbits simi-

larly immunized, agglutinated to some extent some of the ten strains of streptococci from dermatitis herpetiformis. One of these ten strains from dermatitis herpetiformis was also agglutinated slightly in normal horse serum. None of the control suspensions of streptococci from pemphigus, lupus erythematosus and erythema multiforme was agglutinated by the serum from horse (104), and of these suspensions only one, that from pemphigus, was slightly agglutinated in the pool of serums from rabbits immunized with the streptococcus from dermatitis herpetiformis, whereas each was agglutinated strongly in the serum from the horse and the pooled serums from rabbits which had been immunized with streptococci from patients with the respective disease. A pool of strains from arthritis was not agglutinated by any of the pooled immune rabbit serums or horse serums. Twenty-five other single strains of streptococci isolated from dermatitis herpetiformis gave approximately the same results as the ten recorded in this table.

It is apparent after consideration of the results recorded in Table 3 that enough similarity exists between streptococci from patients with dermatitis herpetiformis, pemphigus, lupus erythematosus and erythema multiforme to produce some cross agglutination in the serums from animals which were immunized for extended intervals with these individual organisms.

Agglutinin-absorption in pooled immune rabbit and immune horse serums. It is shown in Table 4, that by the absorption of the pool of immune rabbit serums with each of five single strains and, in Table 5, that by absorption of the immune horse serum with each of ten heterologous strains of streptococci from dermatitis herpetiformis the agglutinins for twenty strains, which were shown to be agglutinated by these untreated serums, were removed from them.

Absorption of this pool of immune rabbit serums with control strains from pemphigus and arthritis, and absorption of the immune horse serum with control streptococci from arthritis failed to show removal from these serums of the agglutinins for any of the twenty strains of streptococci from dermatitis herpetiformis; the pool of immune rabbit serums and the immune horse serum thus treated acted exactly as did these serums untreated.

Precipitin reactions between immune horse serum and alkaline-saline extracts of the streptococcus. Tests for precipitin reactions between the undiluted immune horse serum and alkaline-saline extracts of each of ten individual heterologous strains of the streptococcus from patients with dermatitis herpetiformis were done.

Each of the ten undiluted extracts tested gave strong precipitin reactions (4+ according to the Rosenow scale (8)) with the undiluted serum from the horse which had been immunized with other strains of streptococci from patients with dermatitis herpetiformis. Negative tests were obtained with the serum from a normal horse and with the serum from a horse which had been immunized with streptococci from patients who had arthritis. Control tests with the serums from three other horses immunized individually with streptococci from patients with pemphigus, lupus erythematosus and erythema multiforme also gave some positive reactions with the above extracts. These reactions, however, were weaker and were graded 1+ to 2+.

Further tests between each of these ten undiluted extracts and the above immune serums (except the anti-arthritis serum) and euglobulin solutions of equal concentration from these serums were done, titrating the whole serums and the euglobulin solutions. Positive reactions were obtained between each of the extracts and the whole serum from the horse which had been immunized with

TABLE 4

Agglutinin-absorption Reactions

Absorption from pooled immune rabbit serums of agglutinins for twenty strains of streptococci isolated from patients who had dermatitis herpetiformis by single strains of streptococci from patients who had the same disease

STREPTOCOCCI FROM PATIENTS WITH:	STRAI NUMBER	NORMAL RABBIT SERUM	POOLED IMMUNE RABBIT SERUMS (1:200)								SALT SOLUTION CONTROL
				Treated with streptococci from patients with:							
			Untreated	Dermatitis herpetiformis (Strain 1)	Dermatitis herpetiformis (Strain 2)	Dermatitis herpetiformis (Strain 4)	Dermatitis herpetiformis (Strain 5)	Dermatitis herpetiformis (Strain 9)	Pemphigus (Pool of nineteen strains) ±102	Arthritis (Pool of six strains) #5418	
Dermatitis herpetiformis (Single Strains).....	21	—	3+	—	—	—	—	—	2+	3+	—
	2	—	4+	—	—	—	—	—	3+	4+	—
	3	—	2+	—	—	—	—	—	2+	2+	—
	4	—	4+	—	—	—	—	—	4+	4+	—
	5	—	4+	—	—	—	—	—	4+	4+	—
	6	—	3+	—	—	—	—	—	3+	3+	—
	7	—	3+	—	—	—	—	—	2+	3+	—
	8	—	3+	—	—	—	—	—	3+	3+	—
	27	—	4+	—	—	—	—	—	3+	4+	—
	10	—	3+	—	—	—	—	—	2+	3+	—
	11	—	3+	—	—	—	—	—	3+	3+	—
	12	—	2+	—	—	—	—	—	2+	3+	—
	13	—	4+	—	—	—	—	—	4+	4+	—
	14	—	4+	—	—	—	—	—	4+	4+	—
	23	—	3+	—	—	—	—	—	2+	3+	—
	26	—	2+	—	—	—	—	—	2+	2+	—
	17	—	4+	—	—	—	—	—	3+	4+	—
	22	—	3+	—	—	—	—	—	3+	3+	—
	19	—	3+	—	—	—	—	—	2+	3+	—
	20	—	4+	—	—	—	—	—	3+	4+	—
Pemphigus (pool of nineteen strains).....	102	—	—	—	—	—	—	—	—	—	—

streptococci from dermatitis herpetiformis in dilutions of 1:1,000 to 1:5,000 and with the euglobulin solution from this serum in dilutions 1:100 to 1:500. Positive reactions were obtained with the control, whole immune serums only in dilutions of from 1:10 to 1:100 and with the euglobulin solutions from these control serums only when these solutions were undiluted or diluted 1:5.

From these results it is apparent that the precipitinogen in alkaline-saline

extracts of each of ten individual heterologous strains of streptococci from dermatitis herpetiformis was strongly precipitated by the precipitins in the serum from a horse immunized with other heterologous strains of streptococci from the same source. These results suggest that the precipitinogens in each of the ten extracts

TABLE 5

Agglutinin-Absorption Reactions

Absorption from immune horse serum of agglutinins for twenty strains of streptococci isolated from patients who had dermatitis herpetiformis by single strains of streptococci from other patients with the same disease

STREPTOCOCCI FROM PATIENTS WITH:	STRAIN NUMBER	NORMAL HORSE SERUM (1:2000)	SERUM OF HORSE 104 IMMUNIZED WITH NINE STRAINS FROM DERMATITIS HERPETIFORMIS (DILUTION 1:2000)													SALT SOLUTION CONTROL
			Untreated	Treated with streptococci from patients with:												
				Dermatitis herpetiformis (Strain 1)	Dermatitis herpetiformis (Strain 2)	Dermatitis herpetiformis (Strain 3)	Dermatitis herpetiformis (Strain 4)	Dermatitis herpetiformis (Strain 5)	Dermatitis herpetiformis (Strain 6)	Dermatitis herpetiformis (Strain 7)	Dermatitis herpetiformis (Strain 8)	Dermatitis herpetiformis (Strain 9)	Dermatitis herpetiformis (Strain 10)	Arthritis (Pool of six strains) #5418	
Dermatitis herpetiformis (Single strains)	21	—	4+	—	—	—	—	—	—	—	—	—	—	4+	—
	2	—	4+	—	—	—	—	—	—	—	—	—	—	4+	—
	3	—	4+	—	—	—	—	—	—	—	—	—	—	3+	—
	4	—	3+	—	—	—	—	—	—	—	—	—	—	3+	—
	5	—	4+	—	—	—	—	—	—	—	—	—	—	4+	—
	6	—	4+	—	—	—	—	—	—	—	—	—	—	4+	—
	7	—	3+	—	—	—	—	—	—	—	—	—	—	3+	—
	8	—	3+	—	—	—	—	—	—	—	—	—	—	2+	—
	27	—	4+	—	—	—	—	—	—	—	—	—	—	4+	—
	10	—	3+	—	—	—	—	—	—	—	—	—	—	3+	—
	11	—	4+	—	—	—	—	—	—	—	—	—	—	4+	—
	12	—	4+	—	—	—	—	—	—	—	—	—	—	4+	—
	13	—	2+	—	—	—	—	—	—	—	—	—	—	2+	—
	14	—	4+	—	—	—	—	—	—	—	—	—	—	3+	—
	23	—	3+	—	—	—	—	—	—	—	—	—	—	3+	—
	26	—	4+	—	—	—	—	—	—	—	—	—	—	4+	—
	17	—	4+	—	—	—	—	—	—	—	—	—	—	3+	—
	22	—	3+	—	—	—	—	—	—	—	—	—	—	3+	—
	19	—	4+	—	—	—	—	—	—	—	—	—	—	3+	—
	20	—	3+	—	—	—	—	—	—	—	—	—	—	3+	—
Pemphigus (Pool of 19 strains #102)		—	—	—	—	—	—	—	—	—	—	—	—	—	—

are identical and constitute presumptive evidence that the different strains of streptococci from which these extracts were obtained are identical in type.

The weak cross precipitin reactions obtained between these extracts and the control immune serums suggest that the streptococci from the three control diseases are related to but distinct from the streptococcus from dermatitis herpetiformis. Further control tests between these alkaline-saline extracts and the

serum from a horse which had been immunized with streptococci from arthritis and the serum from a normal horse gave entirely negative results.

Precipitin reactions between immune horse serums and the specific soluble polysaccharide substance of the streptococcus. Tests for precipitin reactions between the undiluted immune horse serums and the specific soluble polysaccharide substance (hereafter abbreviated to S.S.P.) prepared from each of ten individual heterologous strains of streptococci from patients with dermatitis herpetiformis were performed. Each of the ten S.S.P.'s obtained, in a concentration of 1:5,000 in 0.9 per cent solution of sodium chloride, gave strong precipitin reactions (4+) with the undiluted serum from the horse which had been immunized with streptococci from patients with dermatitis herpetiformis. Negative tests were obtained between these S.S.P.'s and the serum from a horse which had been immunized with streptococci from arthritis and with the serum from a normal horse. Other control tests with the serum from each of three horses which had been immunized with streptococci from pemphigus, lupus erythematosus and erythema multiforme respectively, gave positive reactions with some of the above S.S.P.'s. These reactions were weaker and were graded 1+ to 2+.

Further precipitin tests were done, employing the following procedures: (1) testing the undiluted immune horse serums against each of the ten S.S.P.'s diluted in salt solution up to 1:500,000 and (2) testing the ten S.S.P.'s in concentration of 1:5,000 against the immune horse serums diluted up to 1:50,000. In (1), positive tests were obtained with the undiluted serum from the horse which had been immunized with streptococci from dermatitis herpetiformis and each of the S.S.P.'s in concentrations varying from 1:50,000 to 1:100,000, and in (2), positive tests were obtained between each of the S.S.P.'s (1:5,000) and the serum from this horse diluted 1:1,000 to 1:10,000. In (1), control tests were positive with the undiluted serums from each of three other horses immunized individually with streptococci from pemphigus, lupus erythematosus and erythema multiforme and these S.S.P.'s in dilutions of 1:5,000 to 1:10,000, and in (2), positive tests were obtained between each of the S.S.P.'s (1:5,000) and these three control serums diluted 1:10 to 1:100.

Solutions of euglobulin of equal concentrations were prepared from each of the four immune serums. Precipitin reactions were done between each of the ten S.S.P.'s at a concentration of 1:5,000 and these euglobulin solutions undiluted and diluted 1:10, 1:50, 1:100, 1:500, 1:1,000, 1:3000, and 1:5,000. Positive reactions were obtained between each of the S.S.P.'s and the euglobulin solution from the serum from the horse which had been immunized with streptococci from dermatitis herpetiformis in dilutions of 1:1,000 to 1:5,000. The control euglobulin solutions prepared from the other three immune horse serums gave positive reactions with these S.S.P.'s only when undiluted or when diluted 1:10 or 1:50.

From these results it is apparent that the S.S.P.'s obtained from each of ten individual heterologous strains of streptococci from dermatitis herpetiformis was precipitated by the serum from a horse which had been immunized with other heterologous strains of streptococci from the same source. This suggests that the S.S.P. from each of the ten strains of streptococci is identical and, therefore,

that the different strains of streptococci from which these ten S.S.P.'s were obtained are identical in type. It is also apparent that the S.S.P. from each of these ten strains from dermatitis herpetiformis was precipitated but to a much lesser degree by the serums from three horses immunized individually with streptococci from pemphigus, lupus erythematosus and erythema multiforme. The cross precipitin reactions with the above immune serums suggest that the S.S.P. obtained from streptococci from dermatitis herpetiformis is distinct from but related to the S.S.P. in streptococci from patients with the other three diseases named.

Reduction of the cataphoretic mobility of the streptococcus by immune animal serum. The serum from five rabbits, each immunized with a single strain, and the serum from a horse immunized with a mixture of nine strains of streptococci from dermatitis herpetiformis were shown individually to reduce the mobility of fifteen heterologous strains of streptococci from patients with the same disease as compared with their mobility in a solution of sodium chloride, while these same serums reduced the mobility of control streptococci isolated from patients with pemphigus, lupus erythematosus and erythema multiforme little or no more than did normal serums. The results of these tests parallel exactly those already described in similar tests in the study on pemphigus (2, 3). It is unnecessary to repeat the details here.

Cataphoretic mobility in immune serums from which antibodies had been absorbed. —It was also demonstrated that the mobility reducing action of the immune horse serum on ten heterologous strains of streptococci from dermatitis herpetiformis could be removed by absorption of the antibodies from this serum with other single heterologous strains of the same streptococcus. The detailed figures on these experiments are again omitted because they parallel so closely those obtained and recorded in similar studies on pemphigus (2, 3).

EXPERIMENTS TO ESTABLISH A SPECIFIC RELATIONSHIP BETWEEN PATIENTS WITH DERMATITIS HERPETIFORMIS AND THE STREPTOCOCCUS

Precipitin reactions between cleared nasopharyngeal washings from patients with dermatitis herpetiformis and immune animals serums. Tests for precipitin reactions between cleared nasopharyngeal washings from patients with dermatitis herpetiformis and the immune horse and pooled immune rabbit serums were performed. As is shown in Table 6, positive reactions were obtained in sixty-nine or 81.1 per cent of the eighty-five tests made with the serum from the horse which had been immunized with streptococci from dermatitis herpetiformis. These same washings, when tested with control serums from horses (99, 103) and (105) which had been immunized with streptococci from pemphigus, lupus erythematosus and erythema multiforme respectively, gave positive precipitin reactions in thirty-three (38.7 per cent), twenty-eight (32.9 per cent) and thirty (35.3 per cent) respectively, of the eighty-five tests made. Other control tests with these same washings and the serums from other horses immunized with other types of streptococci gave positive precipitin reactions in a very small percentage of

instances. These washings gave no reactions with normal horse serum. The serum from horse (104) which had been immunized with streptococci from dermatitis herpetiformis gave positive reactions with only one (4 per cent) of the twenty-five tests made with the cleared nasopharyngeal washings from patients with other skin diseases.

TABLE 6

Positive precipitin reactions between cleared nasopharyngeal washings and serums of the horses immunized with the streptococcus from (1) patients who had dermatitis herpetiformis and (2) patients with five other diseases

NASOPHARYNGEAL WASHINGS FROM PATIENTS WITH:	CASES	TESTS	INCIDENCE OF POSITIVE REACTIONS IN SERUMS FROM HORSES IMMUNIZED WITH STREPTOCOCCI FROM PATIENTS WITH:						NORMAL HORSE
			Dermatitis herpetiformis (Horse 104)	Pemphigus (Horse 99)	Lupus erythematosus (Horse 103)	Erythema Multiforme (Horse 105)	Encephalitis (Horse 96)	Arthritis (Horse 94)	
Dermatitis herpetiformis	25	85	69 (81.1%)	33 (38.7%)	28 (32.9%)	30 (35.3%)	9 (10.5%)	3 (3.5%)	0
Other skin diseases: (herpes zoster, pityriasis rosea, lichen planus, etc.)	25	25	1 (4%)	1 (4%)	0	2 (8%)	5 (20%)	1 (4%)	0

TABLE 7

Precipitin reactions between cleared nasopharyngeal washings and pooled serums from rabbits immunized with streptococci from (1) patients who had dermatitis herpetiformis and (2) patients who had three other diseases

NASOPHARYNGEAL WASHINGS FROM PATIENTS WITH:	CASES	TESTS	INCIDENCE OF POSITIVE REACTIONS IN POOLED SERUMS FROM RABBITS IMMUNIZED WITH STREPTOCOCCI FROM PATIENTS WITH:				NORMAL RABBIT SERUM
			Dermatitis herpetiformis	Pemphigus	Lupus erythematosus	Erythema multiforme	
Dermatitis herpetiformis	22	63	48 (76.1%)	28 (44.4%)	25 (39.6%)	26 (41.2%)	2 (3.1%)
Other skin diseases (herpes zoster, pityriasis rosea, lichen planus, and so forth)	21	21	1 (4.7%)	1 (4.7%)	0	2 (9.5%)	2 (9.5%)

In Table 7 it is shown that the results of precipitin reactions between the pooled serums from rabbits immunized with streptococci from dermatitis herpetiformis, pemphigus, lupus erythematosus and erythema multiforme, and from a normal rabbit parallel closely those described above (and recorded in Table 6) between these same washings and serums from horses immunized with the same streptococci.

The results of these tests indicate that the cleared nasopharyngeal washings from the patients with dermatitis herpetiformis contain a precipitinogen which is precipitated in a high percentage of the tests made with the serum from a horse and with pooled serums from rabbits immunized with streptococci from other patients with dermatitis herpetiformis.

Precipitin reactions between blister fluid from patients with dermatitis herpetiformis and immune horse serums. Tests for precipitin reactions were done between the fluid contents of fresh blisters from each of five patients who had dermatitis herpetiformis, and the serums from five horses immunized individually with streptococci from patients with dermatitis herpetiformis, pemphigus, lupus erythematosus, erythema multiforme and arthritis respectively and from one normal horse.

Each of these blister fluids when used undiluted gave positive reactions (4+) when tested with the serum from the horse which had been immunized with streptococci from dermatitis herpetiformis. Positive reactions (graded 1 + to 2+ and in one instance 3+) were also obtained between these specimens of blister fluid and the serums of the horses immunized with streptococci from patients with pemphigus, lupus erythematosus and erythema multiforme. Negative tests were obtained between these blister fluids and the serums from the normal horse and the horse which had been immunized with streptococci from arthritis. By titrating these first four immune serums undiluted against each specimen of blister fluid diluted in 0.9 per cent solution of sodium chloride in series of multiples of two, it was demonstrated that each blister fluid gave positive precipitin tests in greater dilution with the serum from the horse which had been immunized with streptococci from patients with dermatitis herpetiformis than with the control serums.

The fluid from blisters in patients with dermatitis herpetiformis was thus shown to contain a precipitinogen which was precipitated in great dilution by the serum from a horse immunized with streptococci from patients with dermatitis herpetiformis, and in a much lesser dilution by the serums from horses immunized individually with streptococci from pemphigus, lupus erythematosus and erythema multiforme, and not at all by the serum from a horse immunized with streptococci from arthritis nor by the serum from a normal horse.

Precipitin reactions between alkaline-saline extracts of the streptococcus and the serums from patients who had dermatitis herpetiformis. Tests for precipitin reactions between the alkaline-saline extracts of each of ten individual heterologous strains of streptococci from dermatitis herpetiformis were performed. The bacterial extracts and serums were used undiluted. Of these tests 55.6 per cent were positive (graded 3+ to 4+). Only 16.5 per cent of the control tests between each of these extracts and twenty serums from patients with pemphigus, lupus erythematosus and erythema multiforme were positive (graded 1+ to 2+). None of the control tests between each of these extracts and five serums from normal persons were positive.

Precipitin reactions between the specific soluble polysaccharide substance of the streptococcus and the serums of patients with dermatitis herpetiformis. Tests for pre-

cipitin reactions between the S.S.P. (diluted 1:5,000) obtained from each of ten heterologous strains of streptococci from patients who had dermatitis herpetiformis and twenty-five heterologous serums (used undiluted) from patients who had dermatitis herpetiformis were performed. Of these tests 62.3 per cent were positive (graded 3+ to 4+). Only 15.5 per cent of control tests between each of these S.S.P.'s and twenty serums from patients with pemphigus, lupus erythematosus and erythema multiforme were positive (usually graded 1+, occasionally 2+) between each of these S.S.P.'s and the serums from five normal persons were positive.

Reduction of the cataphoretic mobility of the streptococcus by serums from patients with dermatitis herpetiformis. The cataphoretic mobility of the streptococcus from patients with dermatitis herpetiformis was determined in human serums. In Table 8 it is shown that the serum of a patient with dermatitis herpetiformis (case 83) markedly reduced the mobility of ten individual heterologous strains of streptococci from patients with dermatitis herpetiformis, as compared with their mobility in a solution of sodium chloride. The control serums from three patients (cases 75, 77, and 66) reduced the mobility of these ten strains of streptococci from dermatitis herpetiformis about equally and little more than did the serum from a normal person. Control streptococci from patients with five other diseases had their mobility reduced little or no more by the serum from case 83 than by the serum from a normal person. It is apparent that the serums from the patients with pemphigus (case 75), lupus erythematosus (case 77), and erythema multiforme (case 66) reduced to any great extent only the mobility of the streptococci isolated from patients with the respective disease. The serums from nine other patients who had dermatitis herpetiformis gave results similar to those obtained in case 83 with the same antigens. The results of these tests may be interpreted as further evidence of the identity of the individual strains of streptococci from patients with dermatitis herpetiformis.

Cataphoretic mobility in human serums from which antibodies had been absorbed. —In Table 9 it can be seen that the serums from two patients with dermatitis herpetiformis when absorbed with single heterologous strains of streptococci from dermatitis herpetiformis reduced the mobility of the control and respective streptococci about equally. Each of these serums untreated and each treated with control streptococci markedly reduced the mobility of the respective streptococci and not that of the controls. Similar results were obtained by absorption of other serums from patients who had dermatitis herpetiformis with still other individual heterologous strains of streptococci isolated from patients with that disease. The above-described phenomenon may also be interpreted as evidence of the identity of the individual strains of streptococci isolated from patients with dermatitis herpetiformis.

Intradermal and subcutaneous injection of dead organisms into patients with dermatitis herpetiformis. Suspensions of streptococci, T 4,000, killed by heat, by formaldehyde solution or by hydrochloric acid were injected intradermally and subcutaneously into twenty-two patients with dermatitis herpetiformis. An urticarial, erythematous papule which became vesicular appeared in from eight

to twelve hours at the sites of these intradermal and subcutaneous injections (fig. 2). Large doses of the dead streptococcus from dermatitis herpetiformis when injected subcutaneously into seven patients with dermatitis herpetiformis produced crops of new lesions in areas formerly occupied by lesions, at the sites

TABLE 8

Reduction of the average mobility of ten heterologous strains of streptococci isolated from patients who had dermatitis herpetiformis by the serum of a patient who had dermatitis herpetiformis

STREPTOCOCCI FROM PATIENTS WITH:	STRAIN NUMBER	AVERAGE MOBILITY IN NaCl (MICRONS PER SEC., VOLTS PER CM.)	AVERAGE REDUCED MOBILITY IN SERUMS FROM PATIENTS WITH: (MICRONS PER SECOND, VOLTS PER CM.) (DILUTION 1:320)				
			Dermatitis herpetiformis Case #83	Pemphigus Case #75	Lupus Erythematosus Case #77	Erythema multiforme Case #66	Normal
Dermatitis herpetiformis (Single Strains)....	1	2.17	0.88 (59%)*	1.78 (18%)	1.50 (30%)	1.64 (24%)	1.89 (13%)
	2	2.20	0.88 (60%)	1.70 (23%)	1.64 (25%)	1.60 (27%)	1.74 (21%)
	8	1.92	0.75 (89%)	1.27 (33%)	1.20 (37%)	1.22 (36%)	1.50 (22%)
	22	1.35	0.63 (53%)	0.99 (17%)	1.14 (15%)	1.16 (14%)	1.20 (11%)
	26	1.84	0.64 (65%)	1.36 (26%)	1.28 (30%)	1.30 (29%)	1.45 (21%)
	30	1.63	0.80 (51%)	1.32 (19%)	1.29 (21%)	1.27 (22%)	1.35 (17%)
	31	1.58	0.84 (47%)	1.26 (20%)	1.22 (23%)	1.26 (20%)	1.32 (16%)
	35	1.49	0.58 (61%)	1.28 (14%)	1.23 (17%)	1.26 (15%)	1.31 (12%)
	37	3.13	0.69 (78%)	2.37 (24%)	2.37 (24%)	2.31 (26%)	2.50 (20%)
	38	1.43	0.63 (56%)	1.18 (17%)	1.21 (15%)	1.15 (19%)	1.22 (14%)
Pemphigus (pool of eleven strains).....		2.32	1.57 (32%)	0.89 (62%)	1.47 (36%)	1.46 (37%)	1.94 (16%)
Lupus erythematosus (Pool of seven strains)..		1.61	1.62 (+1%)	1.60 (1%)	0.71 (56%)	1.53 (5%)	1.81 (12%)
Erythema multiforme (Pool of four strains)....		1.95	1.60 (17%)	1.65 (15%)	1.62 (16%)	0.97 (50%)	1.80 (7%)
Encephalitis (Pool of six strains).....		1.99	1.68 (16%)	1.71 (14%)	1.68 (16%)	1.75 (12%)	1.84 (8%)
Arthritis (Pool of six strains.....		2.02	1.96 (3%)	2.05 (+1%)	2.00 (1%)	1.91 (5%)	2.12 (+5%)

*Figures in parentheses represent the average reduction of mobility, expressed in per cent, under the average mobility in the salt solution control.

of previous intradermal injections of specific vaccines and in previously uninvolved areas.

Control intradermal injections of dead streptococci from patients with pemphigus, lupus erythematosus, erythema multiforme and other cutaneous and systemic diseases produced only areas of slight erythema without urticaria or vesicle formation in a few of these patients with dermatitis herpetiformis. Intradermal injections of the dead streptococci from dermatitis herpetiformis into

patients with pemphigus, lupus erythematosus and erythema multiforme produced, in some instances, small areas of erythema but never bullae.

Intradermal antiserum test. Intradermal injections of whole serum from a horse which had been immunized with nine heterologous strains of streptococci from dermatitis herpetiformis into seven patients who had dermatitis herpetiformis, produced the characteristic erythema-edema (EE) reaction described by

TABLE 9

Removal from serums from patients who had dermatitis herpetiformis of the mobility reducing action on streptococci from dermatitis herpetiformis by absorption with single heterologous strains of streptococci derived from patients with this disease

STREPTOCOCCI FROM PATIENTS WITH:	STRAIN NUMBER	AVERAGE MOBILITY IN NaCl (MICRONS PER SEC., VOLTS PER CM.)	AVERAGE MOBILITY IN SERUMS FROM PATIENTS WHO HAD DERMATITIS HERPETIFORMIS (DILUTION 1:320) (MICRONS PER SECOND, VOLTS PER CM.)						NORMAL SERUM
			Case No. 80*			Case No. 83			
			Unabsorbed	Treated with streptococci from:		Unabsorbed	Treated with streptococci from:		
				Dermatitis herpetiformis (Strain 2)	Arthritis (Pool 5418)		Dermatitis herpetiformis (Strain 1)	Pemphigus (Pool 102)	
Dermatitis herpetiformis (Single strains)	1	2.17	0.91 (58%)†	1.99 (8%)	1.08 (50%)	0.88 (49%)	1.82 (16%)	1.10 (49%)	1.89 (23%)
	2	2.20	0.88 (60%)	1.98 (10%)	1.00 (55%)	0.88 (60%)	1.94 (12%)	1.03 (53%)	1.74 (21%)
Dermatitis herpetiformis (Pool of nine strains) #103		1.86	0.72 (61%)	1.60 (13%)	0.88 (53%)	0.74 (60%)	1.58 (15%)	0.85 (54%)	1.46 (21%)
Pemphigus (Single strain)	1	2.65	1.96 (26%)	1.88 (29%)	1.75 (34%)	1.97 (26%)	1.90 (28%)	1.83 (31%)	2.15 (19%)
Lupus erythematosus (Single strain)	2	1.92	1.55 (19%)	1.75 (9%)	1.55 (19%)	1.61 (16%)	1.56 (19%)	1.63 (15%)	1.84 (4%)

* Similar results were obtained by absorption of eight other serums from patients who had dermatitis herpetiformis with still other individual heterologous strains of streptococci isolated from patients who had dermatitis herpetiformis.

† Figures in parentheses represent the average reduction of mobility, expressed in per cent, under the average mobility in the salt solution control.

Foshay (9) in each patient tested. Control injections of the serums from a normal horse and from immune horses specific for other unrelated diseases produced no reaction in these same patients. Other control injections of the serums from three horses immunized individually with streptococci from pemphigus, lupus erythematosus and erythema multiforme elicited an EE reaction in these patients who had dermatitis herpetiformis but to only a very small fraction of the degree

of reaction produced by the serum from the horse which had been immunized with streptococci from dermatitis herpetiformis.

Solutions of equal concentration of the euglobulin fraction of the serums from five horses which had been immunized individually with streptococci from patients with dermatitis herpetiformis, pemphigus, lupus erythematosus, erythema multiforme, and arthritis respectively, were prepared. These euglobulin solutions from the serums from the first four horses mentioned above elicited EE reactions in these patients with dermatitis herpetiformis which paralleled exactly those described above for the whole serums from the same animals except that with these solutions the difference in degree of reaction between the specific and nonspecific serums was greater. Control intradermal injections of the whole

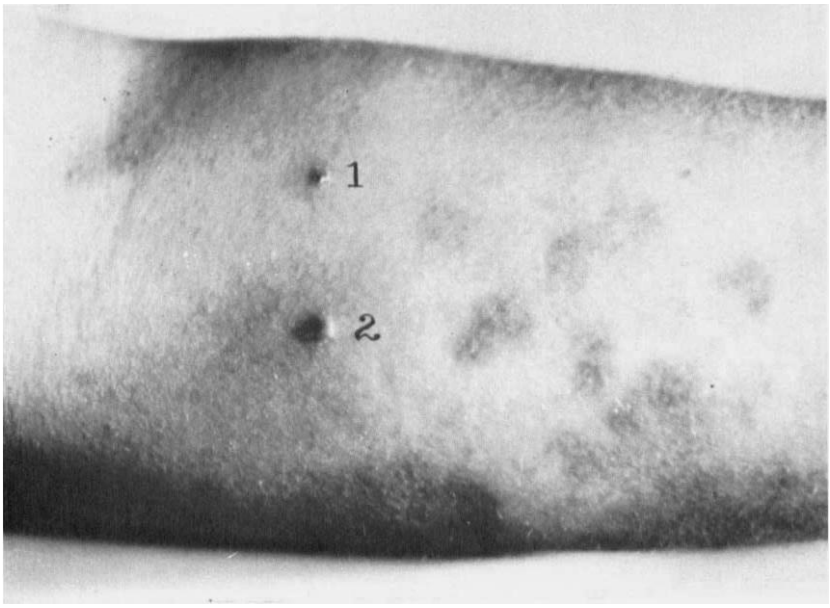


FIG. 2. Lesions 1 and 2 are vesicles produced by intradermal injections into a patien with dermatitis herpetiformis of the specific streptococcus killed by formaldehyde and by heating respectively.

serum and of the euglobulin fraction of the serum from the horse which had been immunized with streptococci from dermatitis herpetiformis into normal persons and patients with unrelated cutaneous and systemic diseases elicited no reaction whatever. From these reactions it would seem that the streptococci from these four diseases are related but not identical in type. Foshay has pointed out that this reaction is a bacterial specific response and wholly unrelated to the phenomenon of serum protein sensitization and also that this reaction has been seen only when a patient with a given bacterial infection has been skin tested with antiserum specific for the infection.

SUMMARY

The morphologic, cultural, staining and fermentation characteristics of a streptococcus which has been isolated from patients with dermatitis herpetiformis

are described. It has been shown to have a characteristic cataphoretic mobility distribution curve and has been found virulent for four species of animals. No attempt has been made to reproduce this disease in animals with this organism.

The different strains of this streptococcus have been shown to be serologically identical through the following procedures: (1) Agglutination reactions between immune horse and immune rabbit serums and heterologous strains of the streptococcus, (2) reciprocal agglutinin-absorption reactions with heterologous strains of the streptococcus, (3) precipitin reactions between immune serums and alkaline-saline extracts of heterologous strains of the organism, (4) precipitin reactions between immune serums and the specific soluble polysaccharide substance of heterologous strains of the organism, (5) the cataphoretic mobility-reducing action of immune serums on heterologous strains of the organism, and (6) reciprocal absorption of the specific mobility-reducing action of immune serums.

A specific relationship between the patients with dermatitis herpetiformis and this streptococcus has been demonstrated by the following methods: (1) Precipitin reactions between the nasopharyngeal washings from patients with dermatitis herpetiformis and the serums of animals immunized with the specific streptococcus, (2) precipitin reactions between the blister fluid from patients with dermatitis herpetiformis and the serum of animals immunized with the specific streptococcus, (3) precipitin reactions between alkaline-saline extracts of the organism and the serums from patients with dermatitis herpetiformis, (4) precipitin reactions between the specific soluble polysaccharide substance of the streptococcus and the serums from patients with dermatitis herpetiformis, (5) the cataphoretic mobility-reducing action of the serums from patients with dermatitis herpetiformis on this streptococcus, (6) absorption by heterologous strains of the streptococcus of this specific mobility-reducing action from the serums from patients with dermatitis herpetiformis, (7) the production of vesicles by intradermal and subcutaneous injection of the dead organism into patients with dermatitis herpetiformis, (8) the production of the erythema-edema (EE) reaction of Foshay in patients with dermatitis herpetiformis by the intradermal injection of immune horse serum.

It has also been shown that streptococci isolated from patients with pemphigus, disseminate lupus erythematosus and erythema multiforme exudativum are similar but not identical in type to the streptococcus from patients with dermatitis herpetiformis.

COMMENT

If the material presented here is to be accepted as factual, dermatitis herpetiformis must be considered a streptococcal disease in which the streptococcus is present on the mucous membranes and in foci. The vesicles and other lesions on the skin must be interpreted as the result of reaction in a sensitized individual to the organism itself or to products of the growth of the organism in that person.

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